

Expression, purification and evaluation of the recombinant human fibroblast growth factor receptor 2b kinase domain

M. Moghadasi*

D. Ilghari**

N. Gheibi***

M. Sirati-Sabet****

F. Khabbaz*****

H. Piri**

*M.Sc. Student of Medical Biotechnology, Qazvin University of Medical Sciences, Qazvin, Iran

**Assistant Professor of Clinical Biochemistry, Qazvin University of Medical Sciences, Qazvin, Iran

***Associate Professor of Biophysics, Cellular and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

****Associate Professor of Biochemistry, Qazvin University of Medical Sciences, Qazvin, Iran

*****M.Sc. in Biochemistry, Qazvin University of Medical Sciences, Qazvin, Iran

*Abstract

Background: Fibroblast growth factor receptor 2b (FGFR2b) plays an important role in cell signaling pathway and regulating several key biological processes including cellular differentiation and proliferation. Genetic alterations (e.g. point mutation in FGFR2b tyrosine kinase domain) are associated with breast cancer, ovarian cancer, and endometrial cancer.

Objective: The aim of this study was to express and purify the recombinant human FGFR2b kinase domain.

Methods: This experimental study was conducted in Qazvin University of Medical Sciences during 2013. Recombinant pLEICS-01 vectors containing the FGFR2b tyrosine kinase domain gene were transformed into E. coli BL21 (DE3). Recombinant protein expression was induced with 1mM IPTG and analyzed by SDS polyacrylamide gel electrophoresis (SDS-PAGE). The recombinant protein was purified by Ni²⁺-NTA affinity chromatography. After dialysis, the protein activity was evaluated by interaction with the wild type and mutant SH2 domains of phospholipase C (PLC) using polyacrylamide gel electrophoresis (PAGE). Data were analyzed using One-way ANOVA and Tukey's test.

Findings: Pre and post-induction SDS-PAGE analysis showed that the expressed protein was soluble at 20 °C. SDS-PAGE analysis also confirmed that no contamination and bacterial proteins were co-eluted with the purified protein. PAGE method results confirmed that the purified protein was in an active state.

Conclusion: With regards to the results, the recombinant FGFR2b kinase domain-a 38 kDa protein-was expressed and purified in an active and soluble state.

Keywords: Fibroblast Growth Factor Receptors, Proteins, Isolation and Purification, Polyacrylamide Gel Electrophoresis

Corresponding Address: Dariush Ilghari, Department of Clinical Biochemistry and Genetics, School of Medicine, Qazvin University of Medical Sciences, Shahid Bahonar Blvd., Qazvin, Iran

Email: d.ilghari@gmail.com

Tel: +98-281-3324971

Received: 11 Nov 2013

Accepted: 7 Jan 2014